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Microbial pelagic metabolism and CDOM characterization in a phytoplankton-dominated versus a macrophyte-dominated shallow lake

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Abstract Dominant primary producer in macrophyte- or phytoplankton-dominated shallow lakes might imply differences in dissolved organic carbon (DOC) composition. We compared chromophoric dissolved organic matter (CDOM), plankton respiration (R), and bacterial (BP) and primary production (PP), in two contrasting shallow lakes. We hypothesized that DOC from the macrophyte-dominated lake would be qualitatively inferior, so that it can support a lower yield than DOC from the phytoplankton-dominated one. Macrophyte-dominated lake had more humic and aromatic CDOM, though molecular weight was similar in both lakes. A clear synchronism between lakes was observed in mean depth and several CDOM absorption coefficients, suggesting an external driver of the variation in DOC concentration and CDOM quality. The positive BP-PP and BP-Chl-*a* correlations in the macrophyte-dominated lake point out to a dependence of bacteria on phytoplankton for a supply of labile DOC. In turn, BP in the phytoplankton-dominated lake was balanced with grazing by HF (heterotrophic flagellates). The significantly higher HB:DOC and HF:DOC carbon ratios in the phytoplankton-dominated lake also suggest that better DOC quality would mean relatively more efficient C transfer to higher trophic levels. According to PP:BP and PP:R ratios both lakes should be considered autotrophic, although the macrophyte-dominated lake would be comparatively more heterotrophic.

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Introduction

In aquatic ecosystems, dissolved organic carbon (DOC) is ubiquitous and plays a central role as main substrate and energy source for heterotrophic bacteria (HB). These prokaryotic microorganisms play a key role in oxidizing DOC to CO_2 through respiration and link carbon transfer to higher trophic levels through predation (Azam et al., 1983).

The main sources of DOC in lake ecosystems are allochthonous inputs of terrestrial material (Aitkenhead-Peterson et al., 2003) and autochthonous primary production (Bertilsson & Jones, 2003). Both carbon sources can be metabolized by HB and act as support for secondary production in many lakes (Karlsson, 2007; Berggren et al., 2010b; Cole et al., 2011). However, the source of DOC and its chemical composition might impact on the efficiency of C utilization by HB. For instance, some evidences suggest that in many lakes bacterial communities consume preferentially and more efficiently autochthonous than allochthonous DOC (Kritzberg et al., 2004, 2005; Kamjunke et al., 2006; Guillemette et al., 2013). Also, the results of previous studies indicate the inefficient transfer of C to higher trophic levels in those ecosystems in which bacteria consume large amounts of terrestrially derived C (Kritzberg et al., 2005; Cole et al., 2006). More recently, a detailed analysis of DOC composition demonstrated that not all allochthonous DOC should be regarded as recalcitrant (Berggren et al., 2010a). Low molecular weight (LMW) DOC of terrestrial origin could be used as efficiently as that of phytoplanktonic origin (Berggren et al., 2010b), although it only made up 3.5% of the total terrestrial DOC (Berggren et al., 2010a). This carbon source, however, could contribute significantly to bacterial growth in those systems with high absolute amount of terrestrial DOC and high total phosphorus concentration (Guillemette et al., 2013).

The early work by Scheffer et al. (1993) showed that shallow lakes could alternate between two distinctive regimes: a transparent one, with low phytoplankton biomass and the presence of macrophytes, and a turbid state, characterized by low transparency, often associated with phytoplankton blooms and the absence of rooted vegetation. Even though, this is probably an excessive simplification, for instance inorganic-turbid lakes (Allende et al., 2009) does not fit in the two categories proposed in this model; macrophyte-dominated and phytoplankton-dominated lakes still represent the most conspicuous scenarios commonly found.

In shallow lakes both, phytoplankton and macrophytes, are important contributors of authocthonous DOC, and thus provide an important subsidy to higher trophic levels (Reitner et al., 1999; Wetzel, 2001; Rooney & Kalff, 2003a; Huss & Wehr, 2004; Lauster et al., 2006). However, the differences in the dominant primary producer in each type of lake imply differences in the quality of autochthonous DOC. Macrophyte-derived carbon is believed to be more refractory to bacterial consumption than phytoplanktonic-derived DOC (Bracchini et al., 2006). Zhang et al. (2013) have demonstrated that under dark conditions, the chromophoric dissolved organic matter (CDOM) produced by phytoplankton was compositionally distinct from macrophyte-derived CDOM. These authors showed that, even though microbial degradation of organic matter of both origins produced labile and refractory fractions of CDOM, there was little change in composition in phytoplankton-derived CDOM during the degradation experiment, while the macrophyte exhibited more qualitative change over time. The authors related these changes to the humification of CDOM molecules along the degradation experiment (Zhang et al., 2013).

In natural environments, the measured DOC is, in fact, the result of a mix of unknown proportions of allochthonous, autochthonous-phytoplanktonic and autochthonous-macrophytic-derived carbon. Nevertheless, and in relation to shallow lakes, there is growing evidence for differences on metabolic processes and carbon cycling in phytoplankton-dominated systems when compared with macrophyte-dominated ones. For instance, Farjalla et al. (2009) observed that highly humic-vegetated lagoons showed proportionally higher bacterial respiration (BR) rates than non-humic lakes, which resulted in low bacterial growth efficiencies (BGE). In agreement with these results, They et al. (2010), in a within-shallow lakes comparison between littoral (dominated by macrophytes) and open water zones (dominated by phytoplankton), suggest that carbon cycling via bacterioplankton may

be more efficient in the open water than in the littoral-vegetated zone as HB biomass, and BP (bacterial production) tended to be higher in the pelagic than in the littoral zone despite lower concentrations of DOC and humic substances. Altogether, these results suggest differences in DOC quality between macrophyte-dominated and phytoplankton-dominated systems, which directly affect bacterial metabolic rates and would affect microbial yields due to differences in C-transfer efficiency through the food web.

The aim of this study was to compare the CDOM and microbial metabolisms in two contrasting shallow lakes. Based in the evidence described above, we hypothesize that DOC quality will be different between the two lakes, less bioavailable in the macrophyte-dominated lake, so that it can support a lower yield than DOC from the phytoplankton-dominated lake. Then, we expect more humic and aromatic DOC, and of higher molecular weight, in the macrophyte-dominated lake; and higher HB biomass, BP, and HF (heterotrophic flagellate) biomass related to DOC concentration in the phytoplankton-dominated lake. In this sense, we performed a synchronous sampling schedule during an annual cycle in a macrophyte-dominated and a phytoplankton-dominated shallow lake from the Pampa Region, and we analyzed comparatively the DOC concentration, CDOM optical properties, and the microbial metabolic rates and biomasses.

Materials and methods

Study site

The study was conducted in two shallow lakes located in the Pampa Plain of Argentina (South America), a warm temperate region with hundreds of naturally eutrophic shallow lakes (Geraldi et al., 2011). We choose two representative lakes with contrasting features. Laguna Chascomús (35°35´S, 58°01´W) is a large shallow lake (area = 30.1 km^2; mean depth ca. 1.8 m) with scarce emergent macrophytes on the shore and with very turbid waters, i.e., Secchi disk fluctuated between 4 and 28 cm (Torremorell et al., 2007; Allende et al., 2009; Fermani et al., 2013). Laguna El Triunfo (35°51´S, 57°52´W) is smaller and shallower than Chascomús (area = 1.5 km^2; mean depth ca. 0.8 m) with the presence of abundant emergent (*Schoenoplectus californicus*) and submerged (*Ceratophyllum* sp.) macrophytes and with transparent (i.e., Secchi disk usually reaches the bottom) humic waters (Allende et al., 2009; Pérez et al., 2010).

Sampling collection

Sub-superficial water samples were collected monthly (November 2010–December 2011) at a central point of each lake. Water level in El Triunfo decreased drastically toward the end of the study period, making impossible the collection of the last two samples.

Abiotic parameters

Surface water level was measured at the sampling site in El Triunfo and at a gaging station in Chascomús. These values were used to estimate mean lake depth (Z_{mean}) using bathymetric charts (Dangavs, 1976). Routine measurements of water temperature, pH (Orion pH-meter), conductivity (Hach conductimeter), and dissolved oxygen concentration (YSI 5000 Meter) were performed in situ. Total suspended solids (TSS) and ash-free dry weight (AFDW) were measured after filtration onto weighed precombusted GF/F filters (APHA, 1998). Total alkalinity was determined by Gran's method (APHA, 1998). Dissolved inorganic carbon (DIC) was estimated using the tables of Rebsdorf (1972). Total phosphorus (TP) and, total dissolved phosphorus (TDP) were determined as molybdate reactive P according to standard analytical procedures (APHA, 1998). Total particulate phosphorus (TPP) was calculated as the difference between TP and TDP. Total organic nitrogen (TON) and total dissolved organic nitrogen (TDON) were determined by semi-micro-Kjeldahl method (APHA, 1998). Total particulate phosphorus (TPP) and nitrogen (TPN) were calculated as the difference between total and the dissolved fraction. DOC concentration was determined using a high-temperature Pt catalyst oxidation method (Shimadzu TOC-5000) following Sharp (1993).

Underwater light characterization

Water transparency was determined with the downwelling vertical diffuse attenuation coefficient of the

photosynthetic active radiation [Kd (PAR)]. Underwater vertical profiles of spectral (380-750 nm) downward irradiance [E_d (λ)] were performed using a calibrated USB2000 (Ocean Optics) spectroradiometer, which was attached to a fiber optic probe with a CC-3-UV-T cosine corrected diffuser yielding a 180° field of view. The attenuation coefficients were determined from the slope of the linear regression of the natural logarithm of E_d (λ) versus depth (Kirk, 1994). Broadband Kd (PAR) was calculated in the same way by integrating E_d (λ) from 400 to 700 nm for each depth. Visual water transparency was determined with a Secchi disk (Z_{SD}). Nephelometric turbidity (T_n) was measured with a bench-top 2100P turbidimeter (Hach) and calibrated against Formazin liquid standards (Hach).

Optical characterization of CDOM

Absorbance of chromophoric dissolved organic matter (CDOM) was measured from filtered (0.22 µm) water samples. Measurements were performed in 0.01 m quartz cuvettes and compared against ultrapure water blank using a Lambda 35 (PerkinElmer) spectrophotometer (from 200 to 800 nm, at 1 nm intervals). In order to correct for possible offsets due to instrument baseline drift, temperature differences, scattering and refractive effects, the average value between 700 and 800 nm was subtracted from each spectrum (Helms et al., 2008). The CDOM absorption coefficient [a_g (λ)] was then calculated using the following equation (Kirk, 1994):

$$a_g(\lambda) = [2.303A(\lambda)]/r$$

where A (λ) is the absorbance at λ and r is the cuvette path length in meters.

For characterizing DOM, several spectral parameters from CDOM absorption coefficients were assessed. The absorption ratio a_g (250)/a_g (365), called molecular size index (MS), is used to track changes in the relative size of DOM molecules (i.e., the mean molecular weight of DOM). As molecular size increases, a_g (250)/a_g (365) ratio decreases (Peuravouri & Pihlaja, 1997). In addition, the absorption ratio a_g (465)/a_g (665) was reported to be inversely related to CDOM aromaticity (Summers et al., 1987). However, ratios have been shown to be also correlated with molecular size, O:C and C:N atom ratios, carboxyl content, and total acidity (Chen et al., 1977) and, therefore, may be suited as a general tracer of humification.

We also determined different spectral slopes in the UV and visible spectral domain. The spectral slopes reported here for the intervals of 275–295 nm (S_1), 350–400 nm (S_2), and 412–560 nm (S_3) were calculated using linear regression of the log-transformed absorption spectra. Slopes were calculated, for a subset of sample spectra, using both the log-transformed linear regression and nonlinear regression approaches by fitting to a single exponential decay function (Helms et al., 2008), with low variation between methods. Spectral slopes and the ratio of spectral slopes ($S_R = S_1/S_2$) have been reported to provide further insights into the average characteristics of CDOM and then to infer characteristics of DOM (e.g., relative molecular weight/size; composition and photobleaching; source; chemical and biological alteration) (Helms et al., 2008; Zhang et al., 2013). $SUVA_{254}$ was calculated by dividing a_g (254) by the DOC concentration in (mg l^{-1}) and used as an indicator of DOM aromaticity (Weishaar et al., 2003). The absorbance at 440 nm, a_g (440), was used as a proxy of the water color (Rasmussen et al., 1989), and the ratio a_g (440)/Chl-a was used as an indicator of lake allochthony (Carpenter et al., 2005).

Microorganism abundance and biomass

Phytoplanktonic chlorophyll-a (Chl-a) was measured spectrophotometrically from water samples (110–250 ml) filtered onto glass-fiber filters (GF/F) after methanol extraction (Marker et al., 1980). Quantitative samples for picoplankton and heterotrophic flagellates (HF) were preserved with 10% ice-cold filtered glutaraldehyde (1% final concentration). Samples were filtered through 0.2 and 0.8 µm black polycarbonate filters (Osmonics Inc.), respectively, stained with 50 µl of DAPI (0.5 mg ml^{-1}) for 10 min (Porter & Feig, 1980) and then mounted on a microscope slide with a drop of immersion oil for fluorescence (Cargille Laboratories). Due to the high abundance of organisms and high amount of suspended particulate matter in Chascomús, the samples were diluted with distilled water prior to filtering (for details see Fermani et al., 2013).

Samples were inspected at 1,000× magnification using Nikon Eclipse 80i microscope equipped with HBO 50 W lamp, and a filter set for blue light, green

light, and UV excitation. HB were counted under UV light excitation and morphotypes were sorted into single-cell and filamentous (>4 μm length). Length of filaments was measured while counted. Picocyanobacteria (Pcy) and eukaryotic picoplankton (Peuk) were clearly recognizable under blue and green light excitation, due to their characteristic photosynthetic pigments fluorescence (Kemp et al., 1993). Picophytoplankton (PPP) was defined as the sum of Pcy and Peuk. HF were counted under UV and blue light excitation and sorted into four size categories: ≤3, 3–5, 5–10, and >10 μm. A minimum of 25 fields was inspected for HB and Pcy, and 200 for HF.

For the estimation of picoplankton biomass, we used the average single-cell HB, Pcy, and Peuk biovolume (V) (0.053, 0.351 and 1.097 μm^3, respectively) and the average width for filamentous HB (0.38 μm) previously estimated in Chascomús by Kranewitter (2010). Using these estimates and the average length of filaments, the bacterial cell carbon content (C_{bact}) was estimated according to Loferer-Krößbacher et al. (1998) as C_{bact} (fg C $cell^{-1}$) = $218 \times V^{0.86}$. Individual cell carbon content for Pcy was calculated assuming a conversion factor of 230 fg C μm^{-3} (Worden et al., 2004). Whereas, Peuk cell carbon content (C_{peuk}) was estimated following the C:V relationship proposed by Menden-Deuer & Lessard (2000) as: C_{peuk} (pg C $cell^{-1}$) = $0.216 \times V^{0.939}$. HF biovolume was estimated by approximating each size-group category to a sphere. The mean cell volume of each group was converted to carbon assuming a conversion factor of 0.22 pg C μm^{-3} (Børsheim & Bratbak, 1987). Average cell carbon contents were 17, 508, 81, 236, and 17018 fg C $cell^{-1}$ for single-cell HB, filamentous HB, Pcy, Peuk, and HF, respectively.

Metabolic measurements

Primary production

Primary production vs. irradiance curves (P vs. I) were obtained using the ^{14}C technique (Steeman-Nielsen, 1952). Lake water aliquots were placed in 16 quartz tubes (45 ml) and each tube was inoculated with 1 μCi labeled sodium bicarbonate. Incubations were performed during 2 h around noon, inside a water bath. The temperature of incubations was the same as that found in lakes. PAR (photosynthetic active radiation) irradiance was measured with an IL1700 (International Light Inc.) radiometer located in the IIB-INTECH (Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús) (35°37′S; 57°59′W). For each incubation series, eight different light intensities (transmittance ranging from 100 to <2%) were set up by covering the tubes with different layers of neutral density filters. In addition, one tube was wrapped in aluminum foil and served as a dark control. All treatments were run with two replicates. At the end of the incubation, 1 ml of each replicate was poured into scintillation vials with 3 drops of concentrated HCl (to estimate total PP); also 1 ml of each replicate was put into scintillation vial with 3 drops of concentrated NaOH (to estimate added activity). The activity was measured in a scintillation counter (Beckman LS 5000TD, Fullerton, CA, U.S.A.) after adding 2.5 ml of OptiPhase 'HiSafe'3 scintillation solution (Holm-Hansen & Helbling, 1995). The photosynthetic parameters for the PAR treatment were estimated from P vs. I curves by fitting the model proposed by Platt et al. (1980):

$$P = P_s * (1 - e(-\alpha * I/P_s)) * e(-\beta * I/P_s),$$

where, P is the photosynthetic rate at a given irradiance (I); P_s is the maximum light-saturated photosynthetic rate; α is the photosynthesis light efficiency at sub-saturating irradiances; β is the negative slope of the curve at high PAR irradiance (i.e., photoinhibition).

Bacterial heterotrophic production

BP was estimated from the rate protein synthesis determined by the incorporation of tritiated leucine into bacterial biomass. Leucine (Leu) was added at saturating concentration (100 nM) to five replicates of 1 ml. Triplicate controls were established with the addition of 100 μl 50% trichloroacetic acid (TCA) before the isotope addition. The Eppendorf tubes were incubated at in situ temperature for 1 h in water baths. The incorporation was stopped with the addition of 100 μl of cold 50% TCA to the Eppendorf. To process the samples, we followed the centrifugation method proposed by Smith & Azam (1992). Finally, 1 ml of cocktail of OptiPhase 'HiSafe'3 scintillation solution was added to the Eppendorf tubes. The activity was measured in a scintillation counter (Beckman LS 5000TD, Fullerton, CA, U.S.A.).

In order to calibrate the methodology, we previously performed curves to determine the incubation time and the concentration of leucine that we should add in each shallow lake. BP (μg C l^{-1} d^{-1}) was estimated assuming the convertion factor of 1.44 kg C mol Leu^{-1} suggested by Buesing & Marxsen (2005) for freshwater. Conversion to daily values was made assuming that BP did not vary over a 24-h cycle.

Leucine-to-carbon conversion factor (CF) experiments

Empirical CFs were estimated at two contrasting seasons [summer (January) and winter (July)], for each shallow lake by mean of dilution experiments (Kirchman & K'nees Hodson, 1985). Briefly, water sample was filtered through 1.2-μm polycarbonate filters (Osmonics Inc.), then diluted (1:9) with 0.2 μm filtered (Osmonics Inc.) lake water, and incubated in 500-ml acid-clean bottles in darkness. The experiment was performed in duplicate. Subsamples were taken every 4 h until bacteria reached the stationary growth phase. H^3-Leucine incorporation rate, bacterial abundance, and biovolumen were measured at each time. HB cell size was estimated by image analysis using a color camera (Nikon DS-Fi1) and following the protocol by Massana et al. (1997). At least 200 cells and 6 images were analyzed from each time and replicate. HB biomass was estimated as explained above. Factors were computed with the cumulative method (Bjornsen & Kuparinen, 1991).

Bacterial grazing

Bacterial grazing by HF (G_{HF}) was calculated following Vaqué et al. (1994, Eq. 1):

$$\mathrm{Log}(G_{\mathrm{HF}}) = -3.21 + 0.99 * \mathrm{Log}(\mathrm{HF}) + 0.028 * \mathrm{T} + 0,55 * \mathrm{Log}(\mathrm{HB}),$$

where HB and HF (cells ml^{-1}) are the abundance of HB and HF, respectively; G_{HF} (HB ml^{-1} h^{-1}) is the grazing rate of HF on HB, and T (°C) is the water temperature. This empirical model assumes that HF grazing is the major contribution to HB mortality. This model was probed to be a fairly good estimation of grazing rates in natural environments (Unrein et al., 2007).

Total pelagic respiration

Total pelagic respiration (R) was determined in vitro from changes in dissolved oxygen using the continuous monitoring of oxygen concentration in a respirometer by means of an oxygen electrode. Water was prescreened in situ through a 45 μm mesh to eliminate the larger, less abundant organisms that were likely to increase the variability of the oxygen consumption.

Continuous measurements were performed with a respirometer chamber (diameter = 13 cm, length = 24 cm, volume = 2.47 l), which was carefully filled with prefiltered water using silicone tubing from the 20 l carboy. The respirometer chamber was incubated in darkness at the in situ temperature. Oxygen concentration and temperature were continuously recorded during the experiments at 1 s interval using a digital oxygen meter (YSI Model 5000) attached to a portable computer. The sensor was tightly introduced into the chamber in order to prevent air exchange. Oxygen consumption rate was estimated from the lineal slope of a plot of oxygen concentration vs. time and expressed as mg O_2 l^{-1} h^{-1}. Oxygen consumption was converted into carbon units using a RQ of 1 (0.375 mg O_2 mg C^{-1}). Conversion to daily values was made assuming that R did not vary over a 24-h cycle. Respirometer chamber was incubated between 4 and 5 h in order to ensure a significant decrease of oxygen concentration (Briand et al., 2004). Differences in oxygen concentration between zero and final time averaged 0.7 mg l^{-1} O_2. Little change of temperature (<1°C) was observed along the incubation. Independent tests with MilliQ water showed that the consumption of oxygen by the electrodes themselves was negligible. Following Briand et al. (2004) and because of the logistic difficulties with one single respirometer system, no replicates were used. Results obtained with two discrete methods (Online Resource 1) did not differ significantly from the continuous method (Wilconxon rank test, $P > 0.05$), giving us confidence to our results.

Statistical analyses

Pearson product-moment correlations were applied in order to investigate the relationship between different variables. When normality was not achieved,

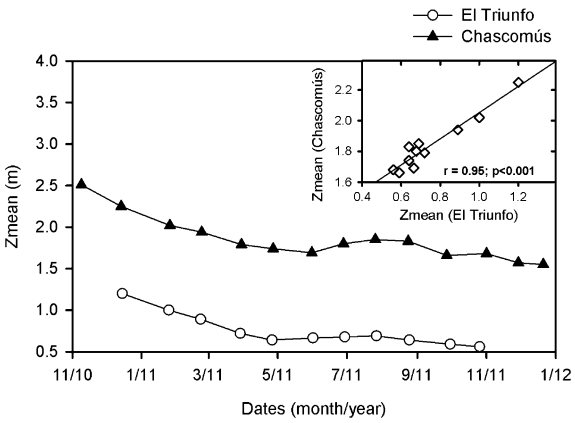

Fig. 1 Temporal variation in mean lake depth (Z_{mean}). *Upper panel* shows the correlation of Z_{mean} between lakes

Spearman rank order correlation was used instead. All statistical analyses were accepted as significant at a probability level of $P < 0.05$. One-way ANOVA tests (using the Holm-Sidak method for multiple comparisons) were carried out to analyze differences in studied variables between lakes. Prior to each analysis, the Shapiro–Wilk and Levene Tests were run in order to test the data for normality and constant variance, respectively. Whenever the data did not conform, Kruskal–Wallis ANOVA on Ranks was utilized (Sigmaplot).

Results

Abiotic parameters

During the whole study period, both lakes exhibited a continuous decreasing trend in Z_{mean}, showing a high synchronism between lakes (Fig. 1). This trend was reflected as an increase in conductivity values in both lakes throughout the study period (data not shown). El Triunfo lake showed significant higher conductivity than Chascomús lake (Table 1). DO concentration evidenced a clear seasonal pattern with minimum in summer and maximum in winter, and it was negatively correlated to water temperature ($r = -0.78$, $P < 0.001$ for the entire data set). DO values were significantly higher in Chascomús lake (Table 1) whereas pH remained above 8.3 in both lakes.

TP concentrations were high in both lakes, although in Chascomús lake was significantly higher than in El Triunfo lake (Table 1). Regarding the different fractions comprising TP, important differences were observed between lakes. While in Lake Chascomús the particulate fraction (TPP) contributed to 80% of TP, in Lake El Triunfo the dissolved fraction (mostly organic) was the most important one, representing on average 58% of TP (Table 1). TON was very high in both lakes, while the dissolved organic fraction (TDON) represented on average 66–70% of TON in both cases (Table 1).

During the entire studied period, we observed clear differences in the underwater light characteristics and suspended solids content between lakes. Lake Chascomús exhibited significantly higher values of TSS, with a lower AFDW content, than El Triunfo (Table 1). The highest values of *K*d (PAR), *T*n, and lower Z_{SD} (Table 1) were registered in Chascomús lake. Also, we observed a clear temporal variation, with less transparent conditions in late-spring (data not shown). In this lake, *K*d (PAR) was inversely correlated with Z_{SD} ($r = -0.78$, $P < 0.001$) and positive with *T*n and TSS ($r = 0.98$ and 0.97, respectively, $P < 0.001$). These correlations were not significant for El Triunfo lake.

Characterization of DOC

The vegetated lake El Triunfo showed a significant c. a. threefold higher DOC concentration than Chascomús lake during the entire studied period (Fig. 2; Table 1). No clear temporal trend was observed; however, both Lakes presented a significant increase of DOC concentration with decreasing water column Z_{mean} (Fig. 3).

Differences between lakes were also appreciable in the optical properties of the CDOM. During all the studied period, El Triunfo presented significantly higher absorption values in the visible [a_g (PAR) and a_g (440)] and UV domains [a_g (365) and a_g (250)] than Lake Chascomús (Fig. 2; Table 1). Concerning temporal variation, both lakes presented a decreasing trend on CDOM absorption coefficients from December of 2010 to July of 2011, followed by a moderate increase during the rest of the year (Fig. 2). These changes were correlated with variations in Z_{mean} (Fig. 3).

The MS index [i.e., the absorption ratio a_g (250)/a_g (365)], inversely related to the molecular weight of CDOM, showed very similar values in both lakes during all the studied period with a sustained increase observed toward the end of the study (data not

Table 1 Average values (Avg.), standard deviation (SD), number of samples (*n*), and range (maximum and minimum values) of all abiotic parameters and DOC and CDOM characterization in both lakes during the study period

		El Triunfo					Chascomús					*P* value
		Avg	SD	*n*	Min	Max	Avg	SD	*n*	Min	Max	
Abiotic parameters												
Z_{mean}	m	0.8	0.2	11	0.6	1.2	1.8	0.3	14	1.6	2.5	<0.001*
Temp.	°C	18.4	6.5	11	9.0	29.0	17.9	6.5	14	6.0	28.0	0.824
Cond.	mS cm^{-1}	2.8	0.7	11	1.9	3.8	1.5	0.3	14	1.0	2.1	<0.001*
DO	mg l^{-1}	8.1	1.9	11	5.5	12.0	9.9	1.9	14	7.2	13.0	0.023*
pH	–	8.8	0.5	11	8.3	9.8	8.9	0.2	14	8.5	9.2	0.197
Alk	mEq l^{-1}	12.9	3.0	12	7.9	17.8	6.6	1.4	14	4.9	10.5	<0.001*
TON	mg l^{-1}	7.995	2.180	12	3.920	12.387	5.647	1.8	13	2.934	8.758	0.007*
TDON	mg l^{-1}	5.692	2.150	12	2.576	9.979	3.499	0.5	13	2.576	4.514	<0.001*
% TDON/TON	%	70	12	12	53	95	66	17	13	42	89	0.549
TP	mg l^{-1}	0.346	0.305	11	0.020	1.099	0.626	0	14	0.148	1.053	0.023*
TDP	mg l^{-1}	0.240	0.313	11	0.004	1.049	0.102	0	12	0.048	0.202	0.255
TPP	mg l^{-1}	0.107	0.099	11	0.002	0.318	0.482	0	12	0.100	0.851	<0.001*
%TDP/TP	%	58	30	11	9	99	20	8	12	6	34	0.002*
TSS	mg l^{-1}	18.7	13	11	5.2	51.6	219.9	117	14	56.0	444.0	<0.001*
AFDW	mg l^{-1}	15.2	10	11	4.5	38.0	73.7	28	14	37.0	130.0	<0.001*
% AFDW/TSS	%	83	9	11	72	100	38	11	14	25	68	<0.001*
Z_{SD}	cm	35	14	5	26	59	10	4	14	6	19	<0.001*
*T*n	NTU	16	7	11	8	31	190	101	14	45	353	<0.001*
K_d (PAR)	m^{-1}	6.9	3	9	1.4	10.0	23.2	9	14	10.3	39.7	<0.001*
DOC and CDOM characterization												
DOC	mgC l^{-1}	71.7	10.4	10	50.5	87.7	22.5	2.6	13	18.5	26.5	<0.001*
a_g (PAR)	m^{-1}	4.3	1.6	10	2.8	6.8	1.2	0.5	11	0.7	2.0	<0.001*
a_g (250)	m^{-1}	429.4	90.5	10	308.4	599.8	102.0	15.5	11	82.4	133.5	<0.001*
a_g (365)	m^{-1}	46.8	14.0	10	31.0	69.3	10.8	3.5	11	7.8	18.7	<0.001*
a_g (440)	m^{-1}	10.7	3.8	10	6.9	16.7	2.8	1.0	11	1.9	4.7	<0.001*
a_g (465)	m^{-1}	6.8	2.5	10	4.3	10.8	1.9	0.7	11	1.1	3.2	<0.001*
a_g (665)	m^{-1}	0.4	0.2	10	0.1	0.6	0.2	0.1	10	0.1	0.3	<0.001*
a_g (250)/a_g (365)	–	9.1	1.3	10	6.5	10.5	9.4	1.4	11	6.8	11.4	0.695
a_g (465)/a_g (665)	–	21.1	8.0	10	13.3	38.3	12.2	3.5	10	8.5	20.7	0.002*
S_1 (275–295 nm)	nm^{-1}	0.023	0.002	10	0.019	0.025	0.022	0	11	0.018	0.025	0.186
S_2 (350–400 nm)	nm^{-1}	0.020	0.001	10	0.018	0.021	0.019	0	11	0.018	0.021	0.025*
S_3 (412–560 nm)	nm^{-1}	0.016	0.001	10	0.015	0.018	0.015	0	11	0.011	0.019	0.031*
S_R	–	1.139	0.044	10	1.035	1.180	1.147	0	11	1.011	1.358	0.751
$SUVA_{254}$	l mg C^{-1} m^{-1}	6.024	1.211	9	4.548	7.940	4.277	1	11	3.032	6.391	0.003*
a_g (440)/Chl-*a*	m^{-1} (µg l^{-1})$^{-1}$	0.255	0.163	9	0.132	0.589	0.011	0	10	0.005	0.029	<0.001*

Significant differences between lakes are indicated with asterisk (*)

Z_{mean} mean lake depth, *Temp* temperature, *Cond* conductivity, *DO* dissolved oxygen, *Alk* alkalinity, *TON* total organic nitrogen, *TDON* total dissolved organic nitrogen, *TP* total phosphorous, *TDP* total dissolved phosphorus, *TPP* total particulate phosphorus, *TSS* total suspended solid, *AFDW* ash-free dry weight, Z_{SD} Secchi depth, *Tn* nephelometric turbidity, K_d *(PAR)* vertical diffuse attenuation coefficient of the photosynthetic active radiation, *DOC* dissolved organic carbon, *ag* absorption coefficient, S_1 S_2 S_3 spectral slopes, S_R ratio of spectral slopes S_1:S_2, $SUVA_{254}$ ratio of a_g (254):DOC

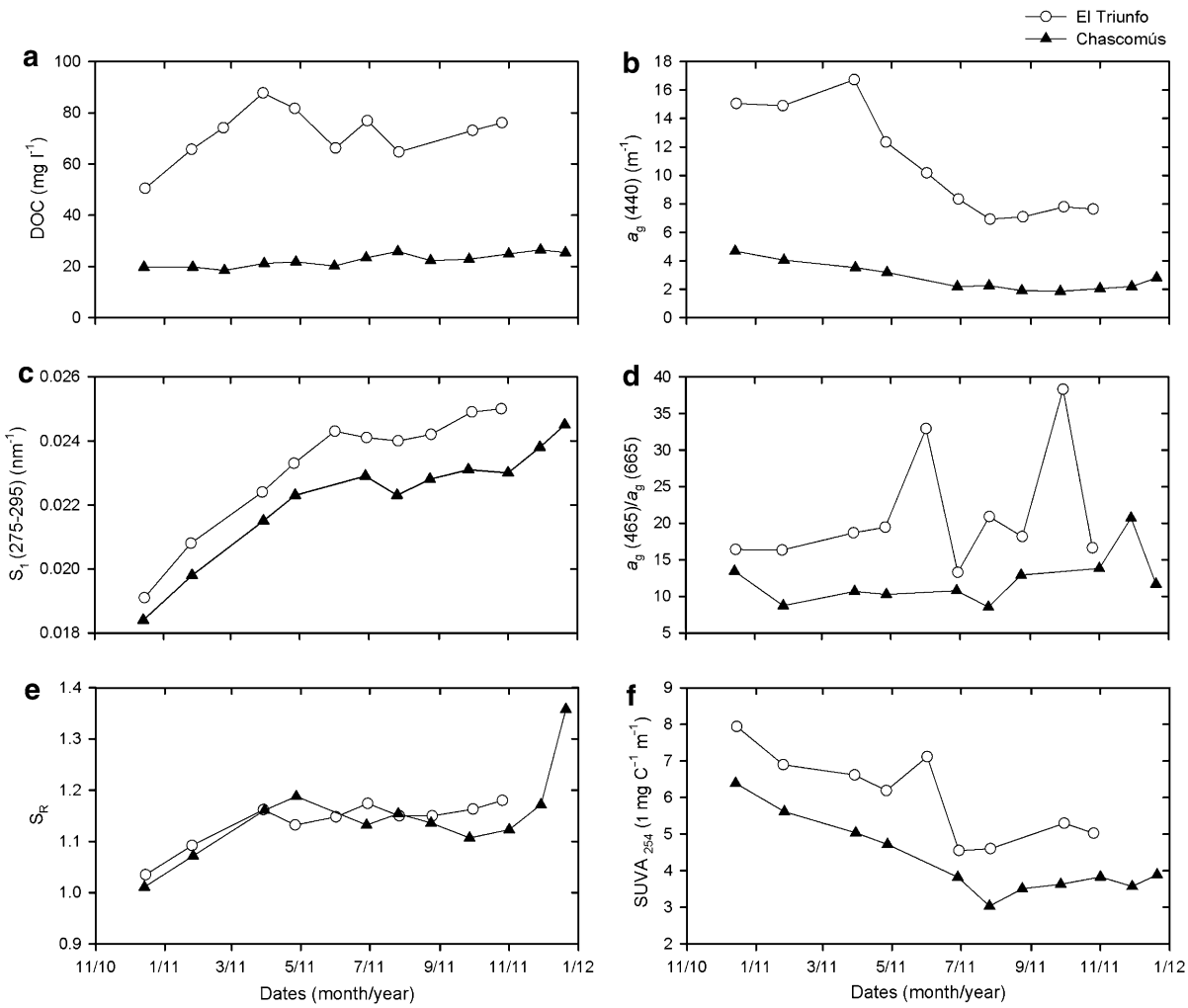

Fig. 2 Temporal variation in **a** dissolved organic carbon concentration (DOC), **b** absorption coefficient at 440 nm [a_g (440)], **c** spectral slope (S_1), **d** absorption ratio 465:665 nm [a_g (465)/a_g (665)], **e** ratio of spectral slopes S_1:S_2 (S_R), and **f** a_g (254):DOC ratio ($SUVA_{254}$)

shown). Values of MS were correlated between lakes ($r = 0.97$, $P < 0.001$). MS index was negatively correlated with the Z_{mean} in both lakes ($r = -0.83$ and -0.96 for Chascomús and El Triunfo, respectively, $P < 0.001$). In contrast, the absorption ratio a_g (465)/a_g (665), inversely related with humification, showed significantly higher values in Lake El Triunfo than in Lake Chascomús during the entire studied period (Fig. 2; Table 1), pointing out a lower humification in the latter. No clear temporal trend could be elucidated for this ratio in any of the lakes.

Spectral slopes S_1, S_2, and S_3, which are inversely related to mean CDOM molecular weight, were higher in El Triunfo than in Lake Chascomús (Fig. 2; Table 1). S_1 was significantly correlated with MS index ($r = 0.88$ and 0.99 for Chascomús and El Triunfo respectively, $P < 0.001$) and negatively correlated with the Z_{mean} in both lakes (Fig. 3). Interestingly, the spectral slope ratio (S_R) between S_1 and S_2, an index of molecular weight and degradation processes, showed minor differences (not significant) between lakes (Fig. 2; Table 1).

The specific absorption coefficient of CDOM at 254 nm (i.e., $SUVA_{254}$), indicative of the percentage of aromaticity, showed significant higher values in Lake El Triunfo (Table 1). During the study, a

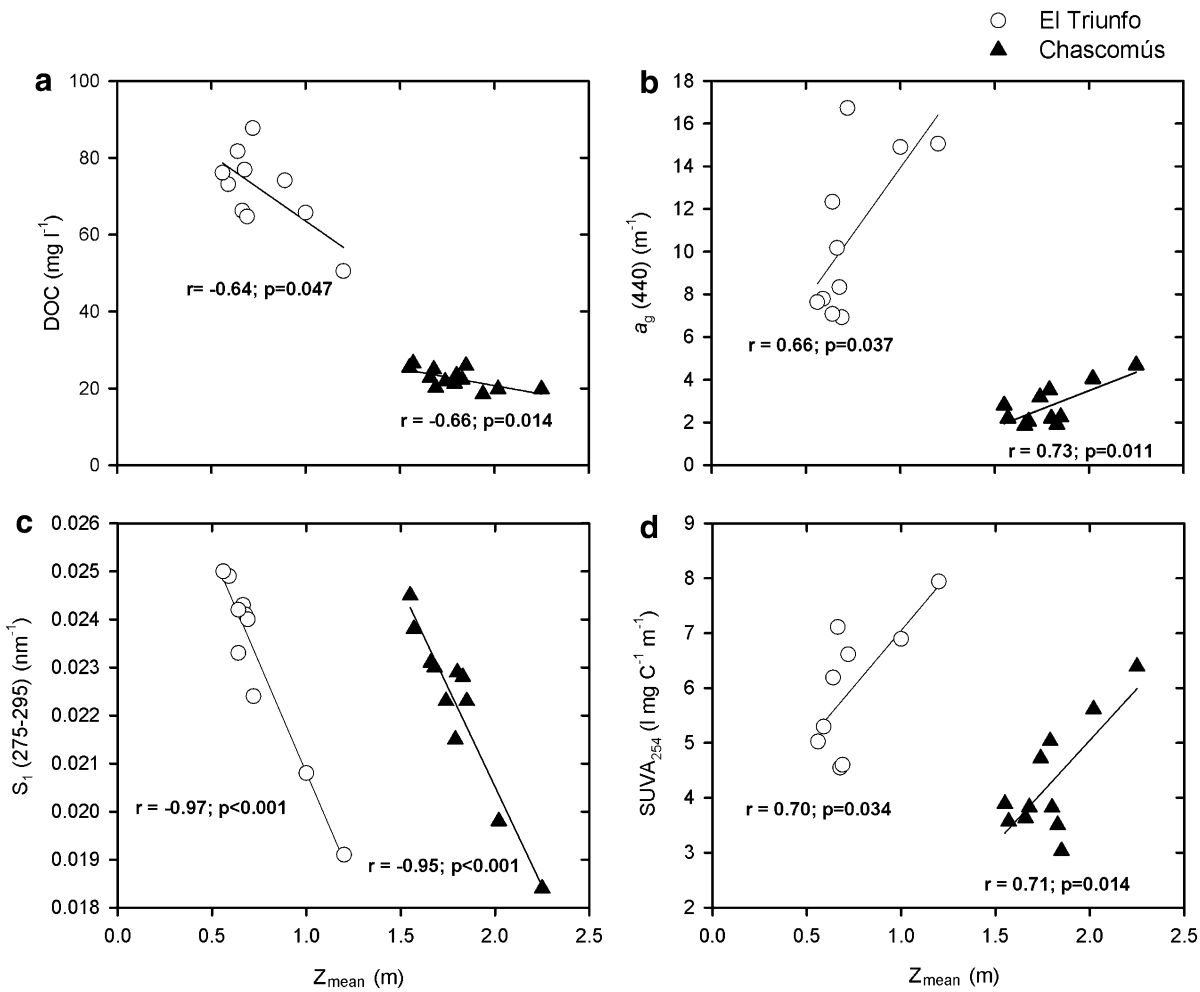

Fig. 3 Relationship between mean lake depth (Z_{mean}) and **a** dissolved organic carbon concentration (DOC), **b** absorption coefficient at 440 nm [a_g (440)], **c** spectral slope (S_1), **d** absorption ratio 465:665 nm [a_g (465)/a_g (665)], **e** ratio of spectral slopes S_1:S_2 (S_R), and **f** a_g (254):DOC ratio ($SUVA_{254}$). When significant, correlation coefficients are shown

decrease in $SUVA_{254}$ was observed for both lakes (Fig. 2). This index was positively correlated with the Z_{mean} in both lakes (Fig. 3). The a_g (440)/Chl-*a* ratio, a ratio usually used as proxy of allochthony, was significantly higher in El Triunfo (Table 1).

Microorganism abundance and biomass

All estimations of planktonic organisms abundance and biomass were significantly higher in the phytoplankton-dominated (Chascomús) than the macrophyte-dominated lake (El Triunfo) (Table 2). On average, HB, HF, and Chl-*a* were four times higher in Chascomús, whereas PPP was more than one order of magnitude higher. Pcy accounted for >90% of PPP. Heterotrophic components of the microbial food web (HB and HF) did not show any clear seasonal pattern, while phytoplankton (Chl-*a*) showed a maximum peak during fall in lake El Triunfo and in late-spring in Chascomús (Fig. 4). In both lakes, Chl-*a* concentrations were closely related to turbidity levels ($r = 0.71$ and 0.78 for Chascomús and El Triunfo, respectively, $P < 0.01$).

Metabolic measurements

Leucine-to-carbon conversion factors (CFs) did not differ between lakes. However, CFs were substantially

Table 2 Average values (Avg.), standard deviation (SD), number of samples (*n*), and range (maximum and minimum values) of microorganisms abundance and biomass, and metabolic measurements in both lakes during the study period

		El Triunfo					Chascomús					*P* value
		Avg.	SD	*n*	Min	Max	Avg	SD	*n*	Min	Max	
Microorganism abundance and biomass												
HB	cell ml^{-1}	1.66×10^7	9.30×0^6	11	3.82×10^6	3.42×0^7	6.44×10^7	1.80×10^7	13	3.37×10^7	9.65×10^7	<0.001*
	µgC l^{-1}	304	164		72	597	1263	352		625	1850	
Pcy	cell ml^{-1}	5.67×10^5	6.64×10^5	11	2.63×10^3	1.8×10^6	7.48×10^6	5.67×10^6	11	2.33×10^6	2.30×10^7	<0.001*
	µgC l^{-1}	46	54		0	150	605	459		188	1860	
Peuk	cell ml^{-1}	1.11×10^4	9.12×10^3	9	1.75×10^3	2.87×10^4	7.73×10^4	6.76×10^4	9	1.38×10^4	1.93×10^5	<0.001*
	µgC l^{-1}	3	2		0	7	18	16		3	46	
PPP	cell ml^{-1}	4.92×10^5	5.56×10^5	9	4.39×10^3	1.54×10^6	7.55×10^6	5.68×10^6	11	2.35×10^6	2.30×10^7	<0.001*
	µgC l^{-1}	41	45		1	126	619	459		193	1866	
HF	cell ml^{-1}	3.79×10^3	3.28×10^3	12	9.64×10^2	1.07×10^4	3.39×10^4	1.61×10^4	12	8.85×10^3	6.05×10^4	<0.001*
	µgC l^{-1}	77	99		6	331	317	189		83	641	
Chl-*a*	µg l^{-1}	76.3	83	12	10.6	318.6	320.6	106.0	14	141.6	475.4	<0.001*
Metabolic measurements												
LIR	nM h^{-1}	23.34	17.08	12	2.48	58.19	13.70	9.63	13	1.06	40.57	0.093
BP	µgC l^{-1} day^{-1}	807	590	12	85.8	2011.1	473	333	13	36.5	1402.0	
PP	µgC l^{-1} day^{-1}	12132	7307	10	5692	23071	12326	5369	14	3541	20029	0.598
R	mgO_2 l^{-1} h^{-1}	0.126	0.101	11	0.024	0.342	0.176	0.100	13	0.050	0.415	0.224
	µgC l^{-1} day^{-1}	1132	912	11	216	3080	1581	900	13	450	3738	
G_{HF}	µgC l^{-1} day^{-1}	27	27	11	4	96	448	274	13	138	1155	<0.001*
Ratios												
HB/DOC ($\times 10^{-3}$)	–	4.4	2.2	10	0.9	8.1	57.3	17.8	13	25.1	81.2	<0.001*
HF/DOC ($\times 10^{-3}$)	–	1.3	1.6	10	0.1	5.1	14.1	8.9	13	3.7	28.7	<0.001*
BP/DOC	day^{-1}	182	259	10	33	887	106	171	12	14	641	0.129
PP/R	–	14.6	11.2	10	3.12	42.20	9.3	5.7	13	3.0	23.4	0.112
PP/BP	–	17.0	12.4	10	8.47	49.20	48.7	50.5	13	4.9	197.0	0.040*

Significant differences between lakes are indicated with asterisk (*)

HB heterotrophic bacteria, *Pcy* picocyanobacteria, *Peuk* picoeukaryote, *HF* heterotrophic flagellate, *Chl-a* phytoplanktonic chlorophyll-*a*, *LIR* leucine incorporation rate, *BP* bacterial production, *PP* primary production, *R* respiration, G_{HF} bacterial grazing by HF, *DOC* dissolved organic carbon

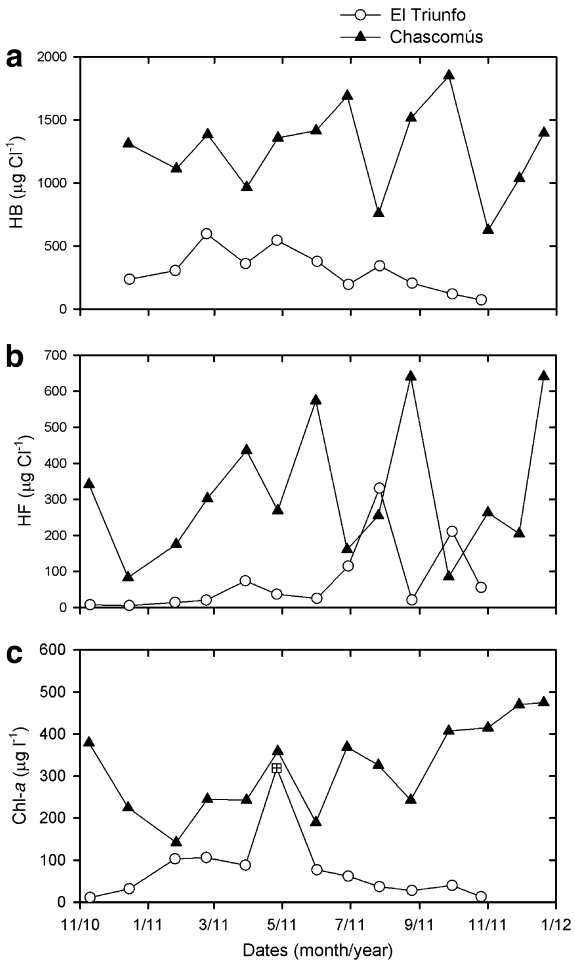

Fig. 4 Temporal variation of plankton biomass: **a** heterotrophic bacteria (HB), **b** heterotrophic flagellates (HF), and **c** phytoplanktonic chlorophyll-*a* (Chl-*a*)

higher in winter (8.88 and 8.78 kgC mol Leu^{-1}, for Chascomús and El Triunfo respectively) than in summer (1.51 and 1.84 kg C mol Leu^{-1}, respectively). Due to the large difference observed between seasons and the lack of estimation of CFs on other dates, we choose a conservative approach and we decided to calculate BP using a consensual published CF of 1.44 kg C mol Leu^{-1}, which is close to the lower value here calculated (see "Discussion" section).

Thus, mean BP was higher in El Triunfo, although no significant differences were observed between lakes (Table 2). A clear seasonality was observed in both lakes with the highest values recorded in January (mid-summer) (Fig. 5). BP was positively correlated with water temperature in El Triunfo ($r = 0.62$, $P < 0.05$), whereas no relationship was observed with DOC concentration. Also, in the macrophyte-dominated lake, BP was significantly correlated with variables related to phytoplankton production (PP) and biomass (Chl-*a*) (Figs. 5, 6). In addition, BP was also positively correlated with HB biomass, while no relationship was observed in the phytoplankton-dominated lake (Fig. 5).

Bacterial grazing by HF (G_{HF}), estimated using an empirical model, was significantly higher in Chascomús than in El Triunfo (Table 2). Mean G_{HF} was roughly similar to mean BP in Chascomús (G_{HF}: BP ratio avg. 1.1), whereas in El Triunfo it only accounted for about 4% of BP.

Mean pelagic respiration (R) did not differ significantly between lakes (Table 2). Higher values of R in Chascomús were recorded during spring-summer and it was significantly correlated with water temperature (Fig. 5). R in El Triunfo did not show any clear seasonal pattern (Fig. 5), though it was significantly correlated with Chl-*a* concentration ($r = 0.65$, $P < 0.05$).

No significant differences in PP were observed between lakes (Table 2). Nevertheless, two different seasonal patterns appeared evident (Fig. 5): in Chascomús higher values were recorded during late-spring in concordance with maximum of Chl-*a* (Fig. 4), whereas maximum PP in El Triunfo were measured in late-summer, similar to BP.

Both PP:R and PP:BP ratios were always >3, but the latter was significantly higher in the turbid than in the clear lake (Table 2). Biomass of HB and HF related to substrate availability (HB:DOC and HF:DOC) were also significantly higher in the phytoplankton-dominated than the macrophyte-dominated lake (Table 2) and were inversely related to the a_g (440)/Chl-*a* ratio (Fig. 7). On average, BP:DOC ratio was higher in El Triunfo, although this difference was not significant (Table 2).

Discussion

Metabolic rates as well as microorganism biomasses were remarkably high in both systems. Despite some differences observed between the phytoplankton-dominated and the macrophyte-dominated lake (see discussion below), values estimated in both water bodies are, though in the upper range, within those recorded for other eutrophic and hypertrophic

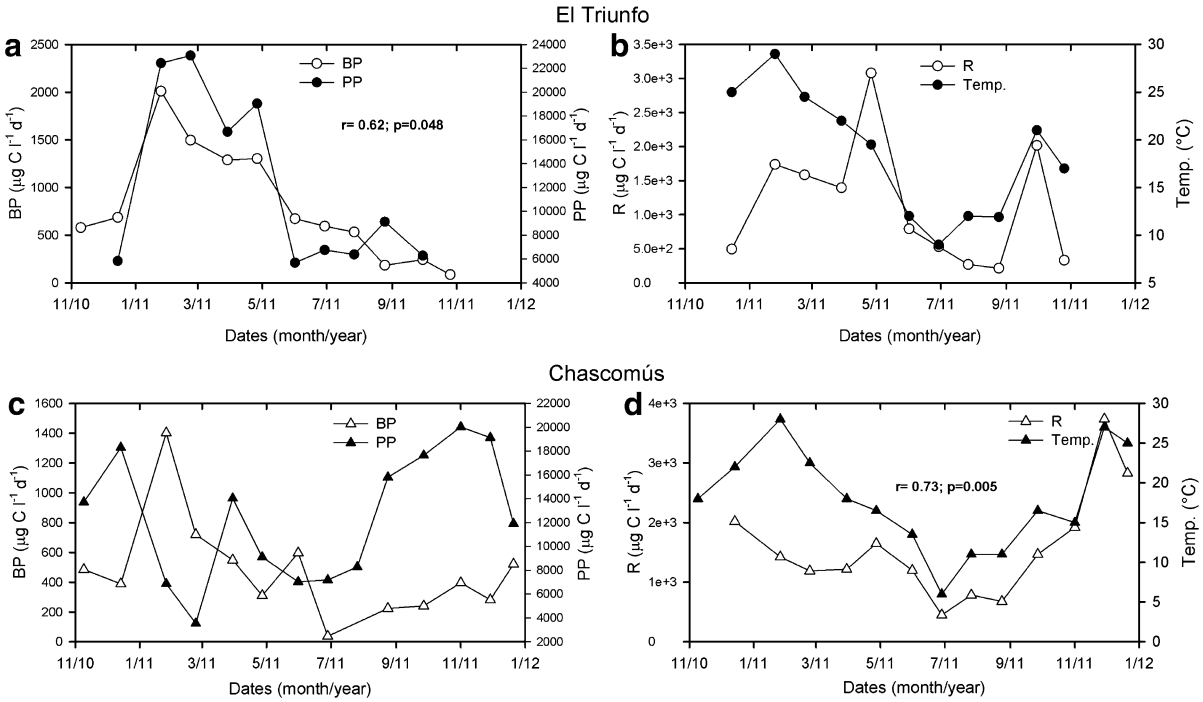

Fig. 5 Temporal variation of **a–c** bacterial production (BP) and primary production (PP), and **b–d** respiration (R) and water temperature (Temp.). When significant, correlation coefficients are shown

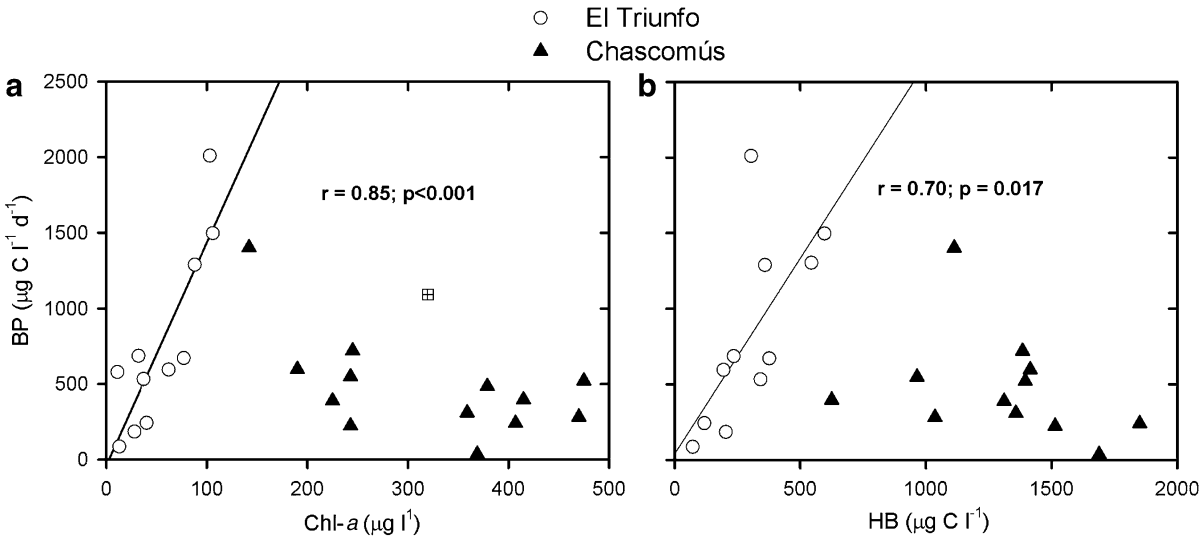

Fig. 6 Relationship between **a** phytoplanktonic chlorophyll-*a* (Chl-*a*) and bacterial production (BP), and **b** heterotrophic bacteria (HB) and BP. When significant, correlation coefficients are shown. Outlier in **a** is shown as a different mark and was omitted from the correlation analysis

shallow lakes (Robarts et al., 1994; Sommaruga, 1995; Kamjunke et al., 1997; Bouvy et al., 1998; Eiler et al., 2003; Rooney & Kalff, 2003b; Waiser & Robarts, 2004; Gao et al., 2007). The leucine-to-carbon conversion factors (CFs) calculated for summer conditions (avg. 1.67 kg C mol Leu^{-1}) were close

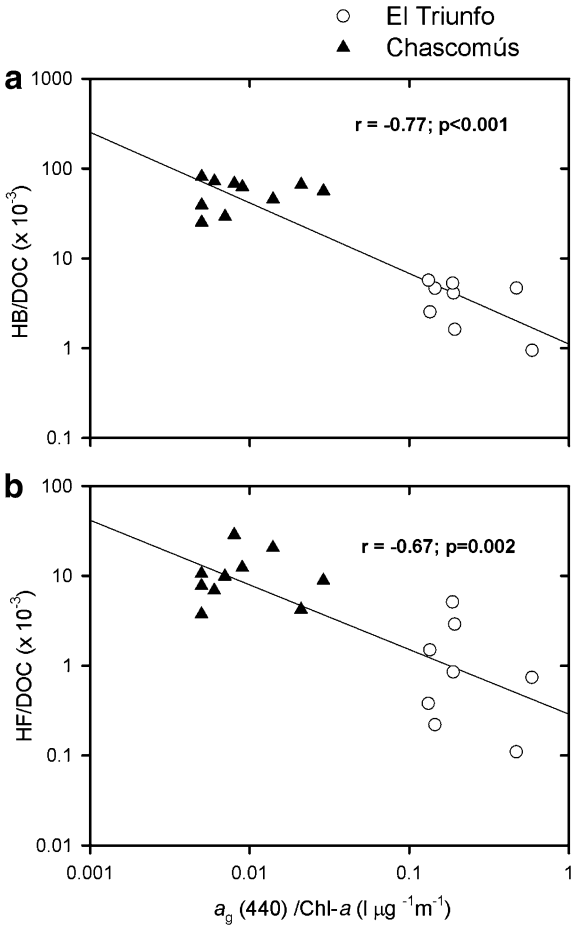

Fig. 7 Relationship between a_g (440):chlorophyll-*a* ratio and **a** heterotrophic bacteria biomass to dissolved organic carbon concentration ratio (HB/DOC), and **b** heterotrophic flagellates biomass to dissolved organic carbon concentration ratio (HF/DOC). Correlation coefficients are shown in each case

to those estimated for other eutrophic shallow lakes (Jørgensen, 1992; Tulonen, 1993; Reitner et al., 1999) and agree with the value recommended by Buesing & Marxsen (2005) to be applied in freshwaters (1.44 kg C mol Leu^{-1}). In turn, values estimated for winter conditions (avg. 8.42 kg C mol Leu^{-1}) were significantly higher and agree with those estimated by Moran & Hodson (1992) in a swamp (8.60 kg C mol Leu^{-1}) and by Baptista et al. (2011) in an estuarine system. It was reported that CFs might vary by a factor of 10, even within the same study (e.g., Sherry et al., 2002; Pulido-Villena & Reche, 2003; Alonso-Sáez et al., 2007). There are also some indications that, in less productive systems, CFs vary in response to DOC quantity, quality, and availability (Pulido-Villena & Reche, 2003), as well as to seasonality (Alonso-Sáez et al., 2008; Calvo-Díaz & Morán, 2009). We are aware that the use of a fixed CF would have resulted in an underestimation of annual BP in both lakes. However, considering the high difference between winter and summer estimations and the lack of figures for other seasons, we chose a conservative approach and applied a standard published CF (Buesing & Marxsen, 2005) that is close to the lower value here estimated.

Throughout the study period, both lakes presented an important decrease of the water column depth. Interestingly, this synchronism in Z_{mean} between lakes was also observed in the temporal variation of several CDOM absorption coefficients, like the absorption ratio a_g (250)/a_g (365), the spectral slope S_1 (275–295), and the $SUVA_{254}$. These trends pointed out to an increase of DOC concentration with the reduction of water column depth, with a concomitant decrease of water color (i.e., a_g (440) values), molecular weight, and aromaticity. Similar trends were already observed for a_g (250)/a_g (365) and conductivity values during a flood event occurred in 2001–2002 in Chascomús (Torremorell et al., 2007). These results suggest an external driver of the variation in DOC concentration and CDOM quality (Pace & Cole, 2002), rather than differences due to contrasting autochthonous sources (i.e., phytoplankton vs. macrophytes). As a result of the evapoconcentration process (largely described by Anderson & Stedmon, 2007), the decrease in water level result in a higher water residence time that could have been accompanied by an increment of the photochemical degradation process, promoting the release of DOC of lower molecular weight, and a colorless situation.

These conclusions could explain the scant differences observed in CDOM quality, i.e., slightly higher values of humification and aromaticity indexes in the macrophyte-dominated lake, in contrast with the clear differences between lakes in DOC concentration and ecosystem states (phytoplankton-dominated vs. macrophyte-dominated). Moreover, it could also explain why we did not observe an increase in BP with the decrease of water column level and the concomitant decrease of CDOM molecular weight in both lakes.

Regarding the phytoplankton-dominated Chascomús lake, we consider that signals related to LMW DOC of better quality produced by the

extraordinary high phytoplankton biomass could not be detected with our characterization of CDOM due to (i) this fraction is comparatively lower in concentration than the DOC of terrestrial origin; (ii) it has a rapid turnover, being always incorporated by the enormous observed bacterioplankton biomass; (iii) it is composed by a high proportion of non chromophoric-dissolved substances (e.g., carbohydrates); (iv) a combination of the above options.

Even though DOC of terrestrial origin is usually consider as recalcitrant, recent evidences suggest that a small proportion of terrestrial DOC is composed by LMW carbon readily available for HB (Berggren et al., 2010a). Thus, LMW terrestrial DOC could significantly contribute to bacterial growth in lakes with high allochthonous DOC concentration (Guillemette et al., 2013), as it could be the case of our studied lakes. Even though we were not able to quantify neither the proportion of each source of DOC nor the preference of HB by each one of them, results of metabolic measurements hinted a higher importance of phytoplankton-derived DOC related to other sources of DOC (either from macrophytes or terrestrial) shaping BP. The following evidences support this idea.

First, it is clear that the observed variations in BP are independent of the DOC concentration. Interestingly, BP follows PP and Chl-*a* in the macrophyte-dominated lake. Despite the high DOC concentration (~70 mg C l^{-1}) measured in this lake, a preference of bacteria for phytoplanktonic-derived DOC may explain the observed coupling between BP and phytoplankton production and biomass in the macrophyte-dominated lake. These results are in agreement with the previous results obtained in net heterotrophic lakes dominated by terrestrial DOC inputs (Kritzberg et al., 2005) and in a series of lakes with increasing submersed macrophytes cover (Rooney & Kalff, 2003b), whereas a coupling between BP and PP was also reported in a prairie lake with high DOC concentration (Robarts et al., 1999). Altogether, these results suggest a dependence of bacteria on phytoplankton for a supply of labile DOC.

Second, even though mean BP was higher in El Triunfo (though not significantly different from Chascomús), mean HB biomass showed the opposite pattern, and figures registered were about fourfold higher in Chascomús (Fig. 6). This result could be explained either by a lower efficiency in the carbon utilization or by a higher grazing pressure in the macrophyte-dominated lake. Our results again point to the first hypothesis. On the one hand, G_{HF} represented only a small proportion of BP in the macrophyte-dominated lake (avg. 4%), indicating that top-down control of HB by HF is unlikely to occur in this lake. On the other hand, the positive correlation between HB biomass and BP could be interpreted as an indication that bacteria are controlled by substrate in a balanced way and, thus, biomass is proportional to production (Billen et al., 1990; Ducklow, 1992; Kisand et al., 1998). Nevertheless, considering the hypertrophic conditions of this macrophyte-dominated lake, limitation by carbon concentration seems unlikely, while limitation by DOC quality could explain the lower figures in HB biomass in the macrophyte-dominated lake. In this sense, limitation of BP by DOC quality was experimentally demonstrated in humic-vegetated lagoons with high DOC concentration (Farjalla et al., 2002). In these lakes, despite the high BP, high bacterial respiration (BR) hints low bacterial growth efficiency (BGE) (Farjalla et al., 2009). Accordingly, Tranvik (1998) showed that, although bacteria grow on isolated humic compounds, the bacterial yield per unit of DOC is higher in the non-humic fraction than in the humic fraction of lake-water DOC. Also in other systems, a positive relationship between BGE-Chl-*a* (Lemée et al., 2002; Alonso-Sáez et al., 2008), BGE-PP (Reinthaler & Herndl, 2005) and a positive coupling between BGE and DOC lability (Middelboe & Søndergaard, 1993; Apple & del Giorgio, 2007) was found, suggesting that BGE could be directly linked to the bioavailability of DOC, and indirectly to PP. Thus, although the high DOC measured in the macrophyte-dominated lake may be an important source of energy for bacteria, it would be not as important as a substrate for bacterial growth since most carbon would be respired.

Even though results are somehow contradictories, patterns in BGE have been also linked with the availability of nutrients. For instance, Smith & Prairie (2004) observed that BGE increase with TDP concentration, while Kritzberg et al. (2010) observe that the effect of phosphate additions on BGE was dependent on the level of BGE, thus BGE was enhanced after P additions only when BGE was lower than 30%. Contrarily, del Giorgio & Newell (2012) did not observe any relationship between BGE and

TDP, though they reported a negative correlation between BGE and labile DOC:TDP ratio, which suggests that DOC quality determine BGE, although this regulation appears to be modulated by nutrient availability. Even though this scenario is unlikely to occur due to the high P concentration measured in our hypertrophic lakes, we cannot discard the potential role of P shaping HB metabolism.

The lack of BP-HB biomass coupling observed in the phytoplankton-dominated lake points out a top-down control on bacteria (Billen et al., 1990; Ducklow, 1992; Kisand et al., 1998). In Chascomús, BP was not related neither to DOC nor to phytoplankton, suggesting no limitation by DOC quantity or quality, while estimation of grazing rates suggest that BP and G_{HF} would be roughly balanced (avg. 473 ± 333 and 448 ± 274 μg C l^{-1} d^{-1}, respectively). The comparison of HB and HF abundances with empirical models (i.e., Gasol 1994) is in agreement with the present results (Fermani et al., 2013, 2014). Even though, Fermani et al. (2013) observed that the degree of HB-HF coupling might be affected by the zooplankton composition, their approach suggest that HB are mostly top-down controlled by HF in lake Chascomús.

On the whole, our results suggest that BP seems to be mostly controlled by the DOC quality in the macrophyte-dominated lake, whereas in the phytoplankton-dominated lake it seems to be mostly controlled by predation by HF.

Differences in DOC quality would imply differences in the efficiency of carbon metabolization by HB, and consequently, in the amount of carbon reaching higher trophic levels (Kritzberg et al., 2005). In line with this reasoning, the biomass of HB and bacterivorous related to bacterial substrate (HB:DOC and HF:DOC ratios) were significantly higher in the phytoplankton-dominated than the macrophyte-dominated lake. Similarly, lower HB:DOC ratios were also observed in a littoral-vegetated lake area in comparison with non-vegetated pelagic waters (They et al., 2010). Interestingly, both ratios were negatively related to the a proxy of DOC quality (Fig. 7), while BP:DOC did not. These results raise the idea that in the macrophyte-dominated lake high amounts of carbon flow through bacteria, but relatively less carbon became finally available to higher trophic levels.

Finally, according to the PP:R and PP:BP ratios, both lakes should be considered autotrophic systems (Jansson et al., 2000; Waiser & Robarts, 2004), which agrees with the expectation for nutrient-rich environments. However, as we expected, in the macrophyte-dominated shallow lake PP:BP and plankton biomass:R ratios were significantly lower than the phytoplankton-dominated one. This is in concordance with the increment of BP and R related to phytoplankton Chl-*a* concentration observed along a series of nine lakes with increasing submersed macrophyte cover (Rooney & Kalff, 2003b). Taking into account that our estimations were restricted to planktonic organisms, it is likely that whole ecosystems' rates were underestimated (Stanley et al., 2003; Lauster et al., 2006). In particular, benthic respiration would theoretically represent a significant proportion of total lake respiration, mainly in the vegetated lake due to its loosely compacted sediment and its large area:volume relationship (Pace & Prairie, 2005). This emphasizes even more the difference between lake types. Overall, these results point out to more heterotrophic conditions, and a relatively higher importance of the heterotrophic pathway in the macrophyte-dominated than in the phytoplankton-dominated shallow lake.

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